

U.S. Patent Application No. 10/697,083

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Human saliva: clinical chemistry and microbiology, Volume 1 By Jorma O. Tenovou

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7. *Practical Aspects Associated with Separation Techniques for Salivary Proteins*

The high molecular weight viscous proteins of saliva pose problems in most analytical or preparative separation techniques. Thus, the viscosity problems concern especially the MSG secretions (see Section II) and submandibular and whole saliva. The viscosity can be decreased by precipitating the salivary mucins with cetyltrimethylammonium bromide,¹²⁴ changing the pH of the saliva by NaOH,¹²⁵ for example, or by mechanical stirring or homogenization of the sample.²⁶ Cetyltrimethylammonium bromide is also widely used as one of the first steps in the purification of salivary glycoproteins.¹²⁶ High molecular weight proteins can also be elegantly removed by ultrafiltration²⁷ using membranes with various exclusion limits.

As can be expected, the methods for preparation of high molecular weight glycoproteins present many practical problems.¹²⁶ In chromatography the high viscosity of saliva leads to broad and overlapping peaks and tailing. Gel permeation chromatography is especially affected by the viscosity of the sample; salivary mucins tend to clog columns. In addition, the columns can be contaminated by insoluble material, for example, by Ca-proteinates present in whole saliva. To avoid these drawbacks, saliva can be filtered through 0.45-µm membranes which also sterilize the samples. Pretreatment of saliva is especially important when using high performance liquid chromatography (HPLC) techniques in which even properly filtered saliva may run a column. Ultrafiltration has appeared to be the most successful method to treat saliva before HPLC. Practical analytical aspects related to the electrophoresis or isoelectric focusing of salivary proteins are discussed in Chapter 7 of this volume.

Further factors interfering with the salivary protein separations include the associations between the salivary proteins and other components of saliva. The equipment used to collect

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